

# HEAT INACTIVATION OF MYCOBACTERIUM PARATUBERCULOSIS IN RAW MILK USING HOLDER-TEST TUBE METHOD AND LAB-SCALE INDUSTRIAL PASTEURIZATION METHOD

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Recent evidence suggests that the etiological agent in Crohn's disease in humans, a severe inflammatory enteritis involving the terminal ileum, may be of mycobacterial origin. Clinical studies have demonstrated the presence of several species of mycobacteria, including *M. fortuitum*, *M. avium-intracellulare*, *M. chelonii*, and *M. kansasii* in intestinal biopsy tissue from Crohn's patients (Chiodini, 1989). More recently, *M. paratuberculosis* has been successfully isolated from patients with Crohn's disease (Chiodini, 1989). Because the clinical symptoms of Crohn's disease closely mimic those found in animals with paratuberculosis, it has been proposed by a number of laboratories that *M. paratuberculosis* may be the causative agent of this disorder.

The advent of molecular biological techniques such as the polymerase chain reaction (PCR) for amplification of DNA combined with the discovery of species-specific genetic probes have greatly improved detection methods for various pathogens. The IS900 insertion element of *M. paratuberculosis* was demonstrated to be a useful probe for detection of this organism in tissue samples. Using this probe to screen intestinal tissues, it was shown that 65% of Crohn's patients (26/40), 4.3% of ulcerative colitis patients (1/23) and 12.5% of controls (5/40) were positive for *M. paratuberculosis* (Sanderson et al, 1992).

Detection of *M. paratuberculosis* DNA was also successful in 72% (13/18) of samples from young children with Crohn's disease with lesser detection in children with colitis and other gastrointestinal disorders (Dell'Isola et al., 1994). The PCR and IS900 probe technology was applied for screening of sections cut from paraffin blocks of Crohn's disease colonic tissue for mycobacterial DNA (Wu et al., 1991). Although designed to detect 30 genomes of mycobacterial DNA including *M. tuberculosis*, *M. kansasii*, *M. avium* and *M. paratuberculosis*, the assay yielded negative results. Other laboratories have also reported a lack of *M. paratuberculosis* DNA in tissues from Crohn's patients (Rosenberg et al., 1991).

The current concerns regarding a possible relationship between Crohn's disease and *M. paratuberculosis* have been stimulated by the recent finding by a group in the U.K. that *M. paratuberculosis* DNA could be detected in pasteurized milk samples purchased from retail markets (Miller et al. 1995). There is no evidence to date that viable *M. paratuberculosis* are present in retail pasteurized dairy products. The present studies were conducted to determine optimal time/temperature conditions for effective killing of *M. paratuberculosis* in experimentally inoculated raw milk. We have compared two methods of heat inactivation, the holder test tube method and the lab-scale pasteurizer method. The holder test tube method has commonly been used by researchers in the past to determine thermal death curves for bacteria. The lab-scale pasteurizer method simulates an industrial pasteurizer unit and closely mimics the high temperature, short time conditions currently used.

Raw milk obtained from *paratuberculosis*-free cows was dispensed into 13 x 100mm snap-cap polystyrene tubes. Tubes were placed in a shaking water bath at either 65, 72, 74 or 76°C with one tube serving as a temperature control. Milk was inoculated with 10<sup>8</sup> CFU *M. paratuberculosis* (Strains 19698, Ben and Ray) and aliquots removed at each time point (0, .25, .5, 1, 5, 15 and 30 minutes) for serial dilution and culture on HEYM for 12 weeks. Results from these experiments demonstrated that *M. paratuberculosis* was effectively killed at

650C [sic] within 15 minutes, however, the holder method of pasteurization (65°C, 30 minutes) is rarely used in the dairy industry today. The most effective reduction in viable bacterial numbers was achieved at 720C [sic] but mean time for optimal killing superceded industry recommendations of 15 seconds at that temperature. Further increases in temperature to 74 or 76°C did not enhance bacterial killing.

Studies with the lab-scale pasturizer were conducted in a similar manner. Raw milk (1-2 liters) was inoculated with three strains of *M. paratuberculosis* at either 104/ml or 106/,1 [sic] final concentration (strains 19698, Ben and Kay). Milk was then poured into the holding vessel and circulated for 15 seconds at 720C [sic]. Samples were obtained from the output tube at the beginning, middle and end of the pasteurization run for serial dilution and culture on HEYM. Results from experiments conducted with the lab-scale pasteurizer demonstrate that treatment of raw milk inoculated with live *M. paratuberculosis* at 720C [sic] for 15 seconds effectively killed all the bacteria. Further experiments evaluating thermal death curves for *M. paratuberculosis* using the lab-scale pasteurizer unit have shown a reduction in bacterial numbers from 106/ml to 103/ml at 55°C, 102/ml at 60°C and complete killing at 65", 70" and 75"C within 15 seconds.

In summary, results using the holder test tube method indicate that *M. paratuberculosis* (108/,1) [sic] were killed after 15 minutes at either 65 or 72°C. Further increases in temperature to 74 or 76°C reduced killing time to between 1 and 5 minutes for the three strains tested. Strain Ben was more heat sensitive than strain 19698 or strain Kay. Using the lab-scale pasteurizer method, treatment of raw milk inoculated with *M. paratuberculosis* at 72°C for 15 seconds killed all bacteria present at all concentrations tested (104 or 106/mi [sic]). Comparison of these two models for testing heat inactivation of *M. paratuberculosis* indicate that the lab-scale pasteurizer more closely simulates industry conditions and that results from those experiments should be given more careful consideration.

## References

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